



# DYNATECH R/D COMPANY

## LIQUID FUELS PRODUCTION FROM BIOMASS

Topical Report: Biomass Substrate Screening for the Liquid Fuels Process  
for

Contract No. XB-0-9291-1

Dynatech Report Number 2136  
Dynatech Project Number SLR-7

### Submitted to:

Dr. Larry Doulgas  
Biomass Refining Program  
Solar Energy Research Institute  
1617 Cole Boulevard  
Golden, Colorado 80401

### Prepared by:

P. F. Levy  
S. R. del Riel  
J. E. Sanderson  
D. L. Wise

May 15, 1981

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Biofuels Information Center

a division of DYNATECH CORPORATION

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DYNATECH R/D COMPANY

A Division of Dynatech Corporation  
99 Erie Street  
Cambridge, Massachusetts 02139  
Telephone: 617-868-8050

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## Section 1

### INTRODUCTION

The basic process under development at Dynatech R/D Company for the production of liquid hydrocarbon fuels consists of three steps. Carboxylic acids are produced from lignocellulosic substrates by mix-culture anaerobic fermentation. Acids are separated and concentrated by liquid-liquid extraction and then converted by electrolytic oxidation (Kolbe electrolysis) to the alkane product.

This report focuses on the anaerobic fermentation of biomass for production of organic acids. Early work on the process used marine algae (particularly Chondrus crispus) as the biomass substrate. Work over the past two years has attempted to expand the applicability of the process to include other biomass forms. The biomass forms tested are representative of three types of potential fermentation substrates. The groups are (1) crop grown biomass such as corn and sweet sorghum, (2) crop residues such as corn stover and wheat straw, and (3) forest products such as pretreated wood chips.

Substrates were analyzed to determine composition (i.e., cellulose, hemicellulose, lignin, ash) and heat content. This allowed predictions of maximum conversions to be made. The cellulose and hemicellulose portions can both be converted to produce organic acids by the bacteria present in these fermentations. The conversion efficiency and rate of conversion of each substrate were measured in static flask fermenters. Residues were recovered and analyzed to determine composition and heat content. Comparison of composition with that of the fresh substrate revealed which component was most easily converted and indicated whether pretreatment of the material was warranted.

In addition to screening the digestibility of a number of substrates, a variety of pretreatments was tested for improvement of conversion efficiencies and rates. A maximum rate was established from the conversion of sucrose in a controlled CSTR-type fermenter. In all cases, rates

appeared to be determined by the solubilization of the substrate by the hydrolytic enzymes produced by the mixed bacterial cultures. This result suggests that pretreatment may be desirable for increasing the rate of conversion and consequently reducing the retention time and digester size. This would have a significant impact on the final product cost since the capital expense of the digesters is the largest equipment cost in the process.

Many of the biomass forms tested appear to be potential process substrates. Some, such as corn stover require a specific pretreatment to make them digestible. Others, such as corn and sweet sorghum, are fermentable directly after harvesting without any further processing. The forest products were converted after pretreatment (by the Iotech process or autohydrolysis) without a hydrolysis step. In all cases rates of conversion could be improved with more extensive pretreatment. This processing alternative should be investigated in the future as a means of reducing retention times in the organic acid fermentation. Future work should also include continuation of alternate substrate testing to expand the base of potential biomass feedstocks for the process.



## Section 2

### PROCESS DESCRIPTION

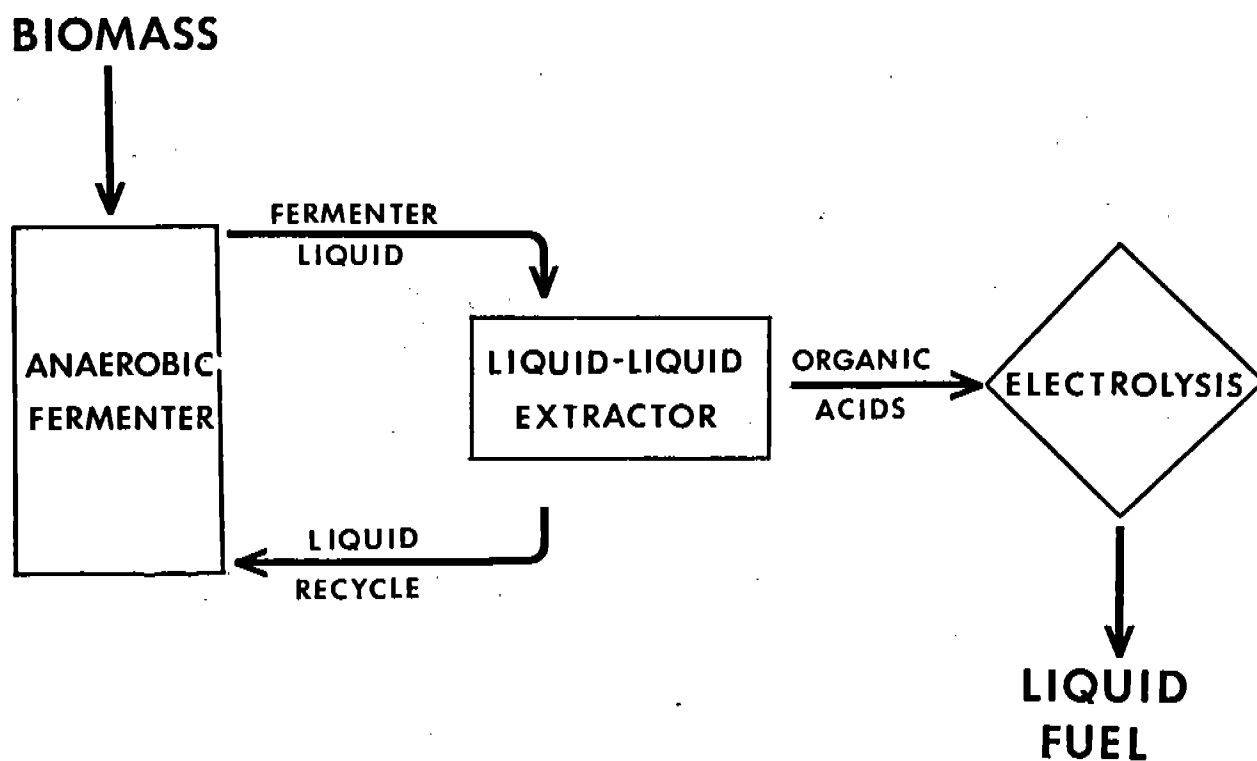
#### 2.1 Process Overview

There are three processing steps involved in the production of liquid hydrocarbon fuel from biomass. Carboxylic acids are produced from polysaccharides by non-sterile anaerobic fermentation. Acids are separated and concentrated by liquid-liquid extraction and then converted by electrolytic oxidation (Kolbe electrolysis) to the alkane product. This process is depicted schematically in Figure 2.1.

Production of organic acids by non-sterile fermentation necessitates the suppression of the methane-forming microorganisms. A wide variety of systems for accomplishing the inhibition of methane formation has been reported in the literature. Tests conducted at Dynatech have successfully employed the specific inhibitor 2-bromoethane sulfonic acid to produce organic acids from a variety of aquatic and terrestrial biomass forms without degradation to methane (Levy et al. 1980; Balch and Wolfe 1976).

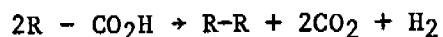
Removal of the product acids from the fermenter is currently accomplished by liquid-liquid extraction. The fermenter broth is circulated through the water-immiscible phase of the extractor. Using kerosene, organic acids are transferred in a second liquid-liquid extractor from the kerosene into aqueous sodium hydroxide. The pH difference between this phase and the fermenter broth provides the driving force for the extraction process.

The monobasic organic acids present in the aqueous sodium hydroxide are electrolytically oxidized to the aliphatic hydrocarbon product, regenerating the sodium hydroxide in the process. This electrolytic oxidation is termed Kolbe electrolysis. Carboxylate is adsorbed on the



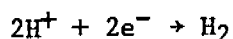
**FIGURE 2.1 | PRODUCTION OF LIQUID HYDROCARBONS FROM BIOMASS**

anode surface and oxidized to form an acid radical. The acid radical decomposes to form  $\text{CO}_2$  and an alkyl radical which can react with another radical to form the Kolbe dimer. Production of the Kolbe dimer,



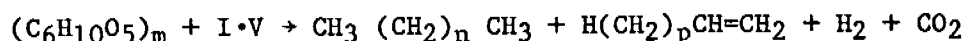
is favored by high concentrations of carboxylate, acidic pH (5 - 6), the absence of foreign anions, high current densities ( $>0.25$  amps/cm<sup>2</sup>), low temperatures, and smooth platinum or iridium anodes (Allen 1958). As side-products in the electrolysis,  $\text{C}_2$  -  $\text{C}_5$  olefins are produced.

As the anode reaction occurs, hydrogen ions are reduced at the cathode, forming hydrogen gas:



The  $\text{H}_2/\text{CO}_2$  mixture produced in the electrolysis can be used to produce electricity in a molten carbonate fuel cell. Operating at 60 percent efficiency and a temperature of  $650^\circ\text{C}$ , the cell will produce sufficient electricity to meet about one-third of the requirements of the electrolysis step. The fuel cell will also produce high quality heat that can be used to make sufficient quantities of high pressure steam to meet all of the pumping requirements of the plant.

In short, the overall process under development may be expressed:



where:  $\text{I}$  = current,  $\text{V}$  = voltage

Previous work at Dynatech has experimentally proven all components of the process. This includes methane suppressed fermentation of biomass to organic acids, recovery of organic acids by liquid-liquid extraction, and conversion of the organic acids to a liquid alkane fuel by Kolbe electrolysis. The present work at Dynatech focuses on the expansion

of the process to alternate substrates (substrate screening) and optimization of the electrolytic oxidation. A lab/pilot scale system is also being operated to provide information for eventual scale-up.

## 2.2 Methane-Suppressed Anaerobic Fermentation

The fermentation employed in the process may be described as a mixed-culture, methane suppressed, anaerobic fermentation whose products are the organic acids acetic through caproic (hexanoic) and carbon dioxide (Sanderson et al. 1979a). It is the higher acids produced (butyric, valeric, and caproic) that are selectively extracted from the fermenter and used to form the dimeric alkane product. The smaller organic acids (acetic and propionic) remain in the fermenter at steady-state concentrations.

The fermentation is similar to methane fermentations used in sewage treatment plants. Inocula are obtained from sewage digester effluent and adapted to digestion of the substrates being tested. A schematic representation of the fermentation process is shown in Figure 2.2. Degradative bacteria use the sugar-containing polymers (cellulose and hemicellulose) in plant material to provide energy for growth. They produce acetic acid,  $\text{CO}_2$ , and  $\text{H}_2$  as their initial metabolic end-products. Since these "acid-forming" bacteria contain cellulolytic enzymes, they are capable of direct use of undissolved polysaccharides. In sewage fermentations, the acetic acid,  $\text{CO}_2$ , and  $\text{H}_2$  are used for growth by methanogenic bacteria which convert these substrates to methane and carbon dioxide.

The fermentation may be altered by inhibiting methane formation. This has been accomplished by using the specific inhibitor 2-bromoethane sulfonic acid (Sanderson et al. 1979b). Figure 2.3 shows the effect of 2-bromoethane sulfonic acid (BES) on organic acid production from the fresh water plant Hydrilla. In the absence of BES, the organic acids produced by the acetogens are degraded to methane and carbon dioxide. The BES eliminates methane production and allows a stable build-up of the organic acid product.

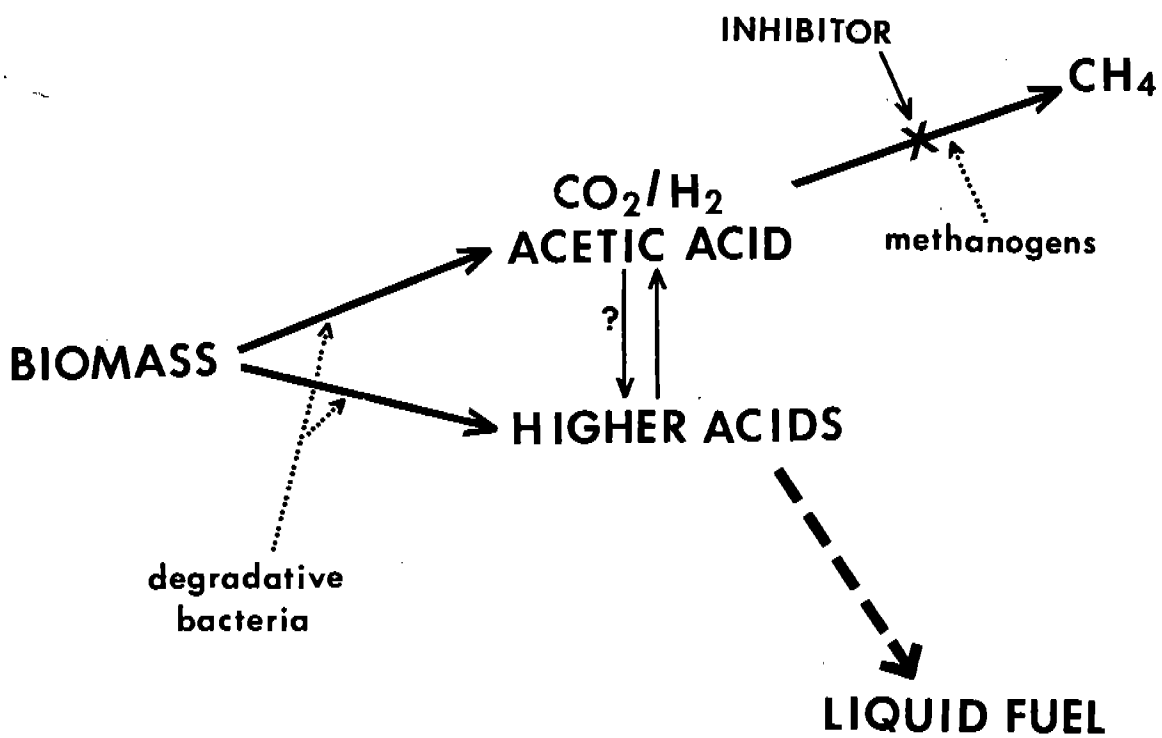


FIGURE 2.2 PATHWAYS INVOLVED IN METHANE FORMATION (BRYANT)

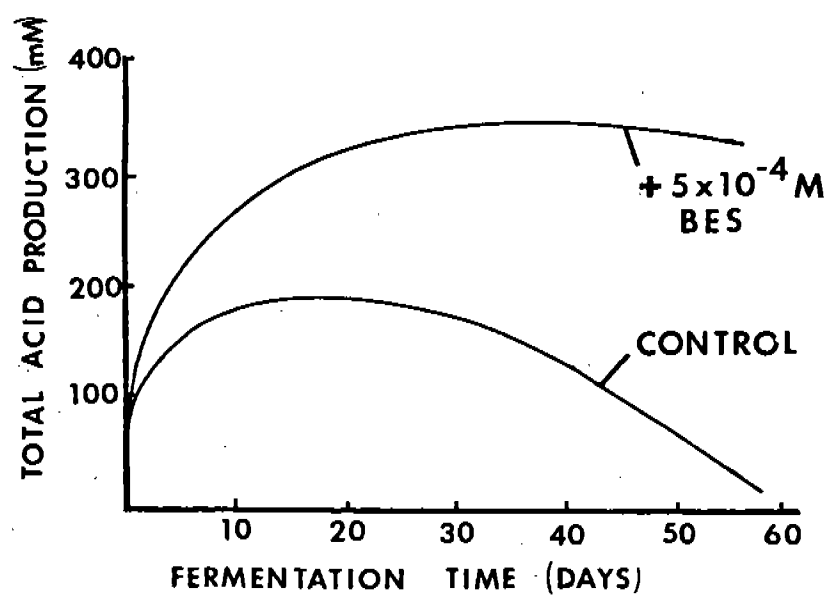


FIGURE 2.3 ) EFFECT OF BROMOETHANE SULFONIC  
ACID (BES) ON ORGANIC ACID PROD-  
UCTION FROM HYDRILLA

As the organic acid level in the fermenter increases and the pH drops, higher molecular weight acids are formed. The  $\text{CO}_2$  and  $\text{H}_2$  may be used to form acetic acid (Ljundahl and Wood 1969) or the  $\text{H}_2$  may be incorporated into the formation of higher acids. The higher acids appear to be formed by addition of 2-carbon units to existing acids. The formation of butyric acid from the condensation of two molecules of acetyl-CoA is hypothesized in Figure 2.4. The increase in butyric acid production in the presence of high acetic acid levels was shown experimentally by adding 100 meq/l of acetic acid to a corn meal fermentation. The results of this experiment, as shown in Figure 2.5, clearly demonstrated the build-up of higher acids by 2-carbon additions. Similar experiments in which propionic or butyric acid levels were elevated showed increased production of valeric or caproic acid.

In the fermentation process being developed, the substrate biomass is converted to organic acids (butyric, valeric, and caproic) which can be removed from the fermenter and converted to a liquid hydrocarbon product. Conditions favoring higher acid formation are acidic pH (4.8 to 5.0), presence of the specific methane inhibitor BES, and high acetate concentration.

### 2.3 Goals of Substrate Screening Experiments

A variety of potential biomass substrates were tested to determine their applicability to the fermentation process described above. Experiments were performed to measure digestibility by mixed bacterial cultures under anaerobic conditions. Analyses of substrates and fermentation residues were also performed to assess maximum potential conversions and determine which portions of the materials tested were most digestible. Rates of conversion and conversion efficiencies were determined from batch data in most cases.

Numerous pretreatments were attempted to increase rates and efficiencies of product formation. These could be compared to rates of untreated materials to evaluate the benefit of the pretreatments. While more work is still required to determine the optimum operating conditions

Figure 2.4 )  
BUTYRIC ACID FORMATION

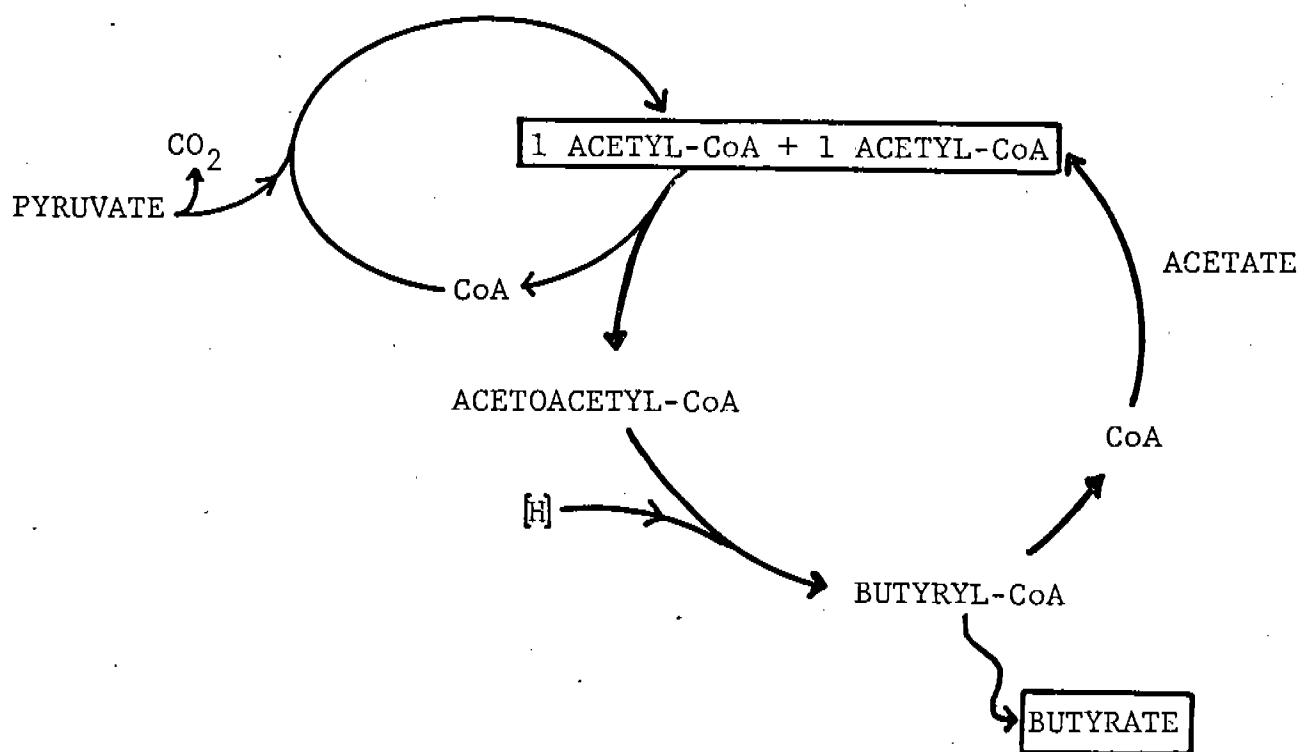
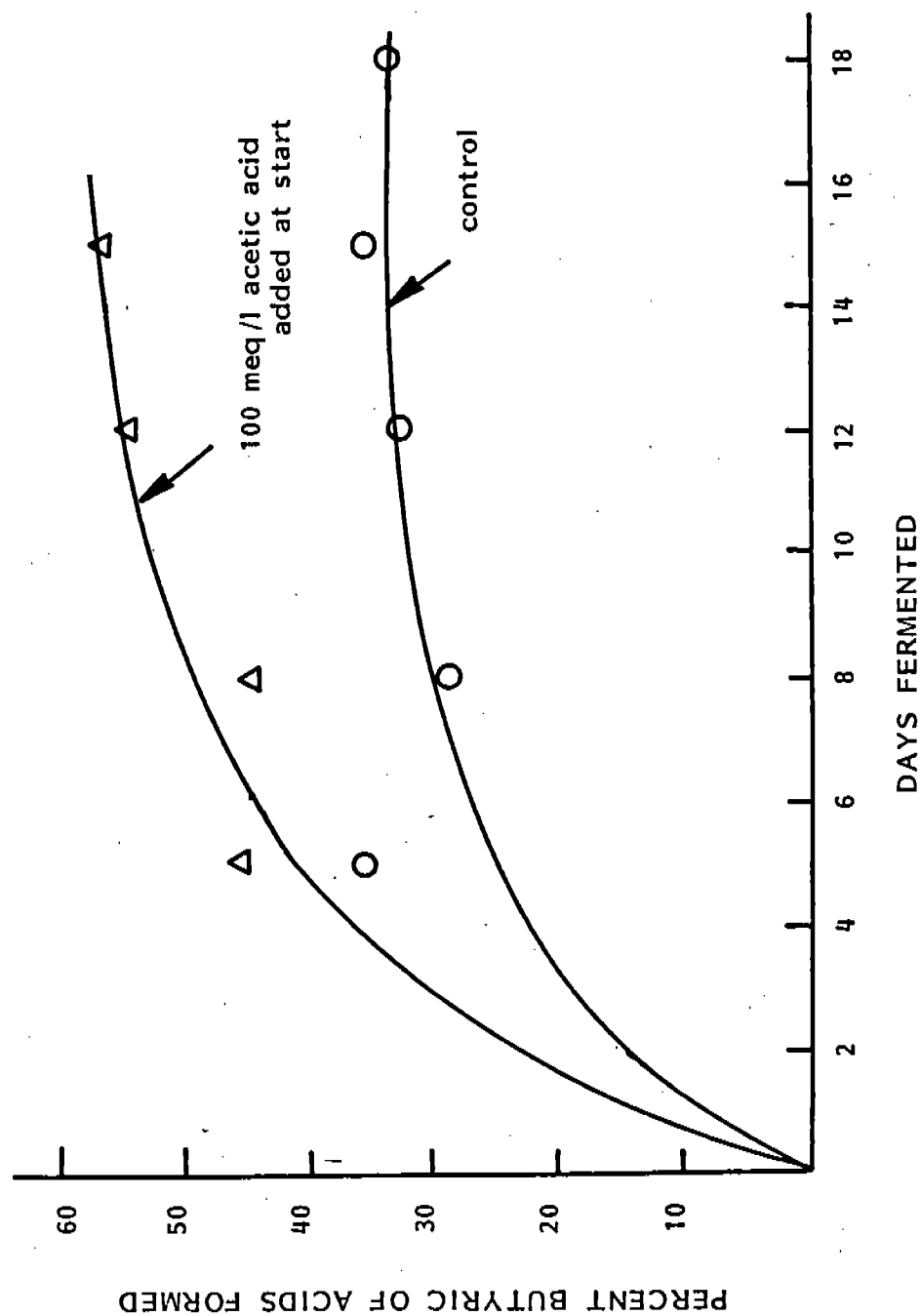




FIGURE 2.5

BUTYRIC ACID FORMATION FROM CORN MEAL



for each substrate in the fermentation, the results reported here have enabled us to establish a rational method for examination of potential biomass feedstocks for the process.

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## Section 3

### SUBSTRATE ANALYSIS - EXPERIMENTAL METHODS

#### 3.1 Digestibility Experiments

Static flask fermentations of potential biomass substrates were routinely performed to measure conversion efficiencies of pretreated and untreated materials. The fermentations were done under anaerobic conditions with mixed microbial cultures initially obtained from sewage digester effluent.

A nutrient medium was used to ensure that bacterial growth was not hampered by lack of essential trace metals or vitamins. The medium contents are listed in Table 3.1. Fermentations have total volumes of 400 ml in 500 ml Erlenmeyer flasks. Each fermenter has a gas sampling port and an effluent gas hose. Effluent gas passes through a trap flask and is bubbled through a water trap. The head space in the system is flushed with  $N_2$  to provide anaerobic conditions when the experiment is started.

The substrate being tested is added to the fermentation at concentrations between 2 and 5 percent based on total solids (i.e., 20g to 50g substrate per liter). Inoculum contains less than 2 percent of the total solids added to the fermenter. Calcium carbonate (1 g/l) is added to the fermentation as buffer, to maintain the pH above 4.5. More calcium carbonate is added as required. Flasks are shaken daily to provide mixing.

Acid production is measured by gas chromatography. Peaks of organic acid products are identified and quantified by comparison with standards. Samples for gas chromatographic analysis are first centrifuged to remove solids and then acidified with sulfuric acid before injection into the gas chromatograph. A column packed with Chromosorb 101® (Johns-Manville, Mesh Size 80/100) is used for the separation of the organic acids with a temperature program ranging from 160 to 230°C.

Table 3.1  
Composition of Nutrient Medium for  
Static Flask Fermentations\*

<u>Compound</u>	<u>Concentration (g/l)</u>
$(\text{NH}_4)_2\text{SO}_4$	2.4
$\text{KH}_2\text{PO}_4$	0.4
$\text{K}_2\text{HPO}_4$	0.4
EDTA	0.008
Yeast Extract (Difco)	0.2
$\text{CaCO}_3$	4.0
$\text{CaCl}_2$	0.04
2-bromoethane sulfonic acid, sodium salt	0.08
trace salts	4.0 ml

Trace Salts

<u>Salt</u>	<u>Concentration g/l</u>
$\text{FeCl}_3 \cdot \text{H}_2\text{O}$	1.67
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.018
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.016
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.033
HCl to pH 2	

---

\* Adapted from Han, Lee, and Anderson 1975. J. Agric Food Chem. 23:928.

The following equations are used to calculate conversions based on organic acid concentrations:

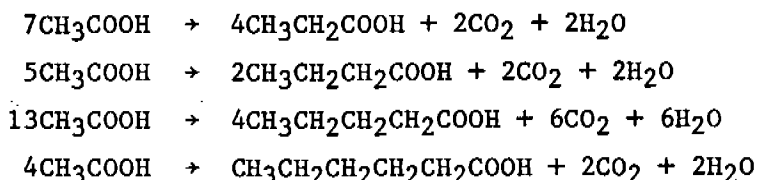
$$C = 0.06 R/S$$

where C is percent conversion, R is total reducing equivalents of product in meq/l, and S is the substrate concentration in g/l, and

$$R = A + 1.75P + 2.5B + 3.25V + 4.0C$$

where A, P, B, V, and C are the measured concentrations of acetic, propionic, butyric, valeric, and caproic acids in meq/l.

This correlation is based on the following set of disproportionation reactions:



The head space is occasionally checked for methane by gas chromatography. If trace amounts are observed, more BES is added to the fermenter broth. All conversions are reported as percent of volatile solids converted to products.

### 3.2 Compositional Analysis of Substrates

Analyses to determine the heat of combustion, ash, lignin, cellulose and hemicellulose, and toluene-ethanol soluble fractions, and to identify the sugars solubilized by hydrolysis were performed on all substrates. This information allowed predictions of maximum conversion to be made.

Heats of combustion were measured in a Parr Bomb Calorimeter® on oven dried samples. The total ash (inorganic salt) content was determined

by burning an oven dried sample at 600°F in a muffle furnace and comparing weights before and after burning:

$$\text{Percent Ash} = \frac{\text{weight after burning}}{\text{weight before burning}} \times 100$$

The portion of material burned in the muffle furnace is referred to as the volatile solids:

$$\text{Percent Volatile Solids} = 100 - \text{Percent Ash}$$

A more detailed analysis to identify the composition of the volatile fraction was also performed. An oven dried sample (3 to 5 g) was weighed in a dried, tared, medium porosity Alundum thimble. The thimble and contents were placed in a Soxhlet apparatus and extracted with 2:1::toluene:ethanol until constant weight was reached. Typically, this required 24 hours of extraction time. The thimble was dried, cooled, and weighed to determine the extractable portion:

$$\text{Percent Extractives} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100$$

The toluene-ethanol extractable portion contained in plant biomass consists mainly of resins. Some soluble sugars may also be extracted in this procedure.

Approximately 0.5g of dry extracted material was accurately weighed into a 3-dram vial. The sample was hydrolyzed with 5.0 ml of 72 percent  $\text{H}_2\text{SO}_4$  at 30°C for one hour with intermittent mixing. The liquor becomes darker and more viscous during this time. The contents of the vial were then quantitatively transferred to a 125 ml Erlenmeyer flask and diluted with 50 ml water, producing a 6.5 percent  $\text{H}_2\text{SO}_4$  slurry. After placing a watch glass over the flask, it was put in a 110°C oven for three hours. The remaining undissolved solids were recovered by filtering through

a dried tared porcelain filtering crucible. The filtered solids were washed with 20 ml water and the crucible and contents dried at 110°C to a constant weight. The cooled crucible was accurately weighed. The solids remaining in the crucible were lignin and non-dissolved ash. The lignin was burned in the 600°F muffle furnace and its weight determined by difference. The percent lignin in the sample after extraction can be calculated:

$$\text{Percent lignin} = \frac{\text{weight after hydrolysis} - \text{weight after burning}}{\text{weight before hydrolysis}} \times 100$$

The hydrolyzed portion (polysaccharides) can also be calculated:

$$\text{Percent hydrolyzed} = \frac{\text{weight before hydrolysis} - \text{weight after hydrolysis}}{\text{weight before hydrolysis}} \times 100$$

These components are calculated as percentages of the hydrolysis sample (above) and then corrected to reflect the pre-extraction percent of total solids.

The identity of the hydrolyzed sugars contained in the liquor was then investigated. The combined filtrate (approximately 70 ml) was neutralized to pH 7 with NaOH, and the final volume recorded. Approximately 12 ml of the neutralized filtrate was filtered through a 0.45µ Millipore HA filter® and 10 ml of this filtrate were added with constant mixing to 40 ml HPLC grade CH<sub>3</sub>CN. Precipitation of salts may be induced by seeding or by gentle heating. The 80:20::CH<sub>3</sub>CN:H<sub>2</sub>O solution was decanted and filtered through a 0.5µ Millipore FH® filter. The filtrate was then analyzed by High Pressure Liquid Chromatography (HPLC) for carbohydrates. Samples (0.4 ml) were injected onto an Alltech 600 CH column (30 cm, 4.1mm i.d., 2µ frit size) with 80:20::CH<sub>3</sub>CN:H<sub>2</sub>O as the moving phase and an elution rate of 2.5 ml/min. Sugar peaks were detected with a Waters Differential Refractometer R401 and identified and quantified by comparison to standards. The quantity of each sugar detected was calculated as percent of hydrolysis sample and corrected to pre-extraction percent of total solids.



### 3.3 Compositional Analysis of Fermentation Residues

The portion of the substrates that was unconverted in each fermentation may be determined by recovering the unconverted solid residue from the fermenter and analyzing its composition. Determination of which sugars are difficult to ferment is helpful in choosing potential substrates and developing pretreatments to improve degradability.

The fermenter residues were recovered by decanting the liquid and then resuspending the solids in water containing HCl (pH 1-2) to dissolve the  $\text{CaCO}_3$ . The solid residue was then separated by filtration, dried in a  $110^\circ\text{C}$  oven, weighed, and then analyzed by the same methods used for fresh substrates (described in Section 3.2). The analyses determine the heat of combustion as well as composition.

### 3.4 Operation of Continuous-Stirred Fermenters

Continuous-stirred fermenters (3-liter liquid volume) have been used to obtain more precise rate data than is available from static flask fermentations. To date, experiments have been performed using sucrose as the substrate to determine the rate constant for conversion of a soluble substance under process operating conditions. Microbial nutrient requirements and doubling times were also determined.

The fermenter was equipped with temperature and pH controllers. The temperature was maintained at  $37^\circ\text{C}$  and pH at  $5.0 \pm 0.2$ . Ammonium hydroxide (10%) was used to adjust the pH and was added by a pump triggered by a pH controller. The fermenter was run in a semi-batch fashion with retention times of 1 to 3 days and sucrose loading concentrations of 20 to 75 g/l. Total organic acid concentrations were maintained at 50-150 meg/l.

The optical density at 640 nm was monitored and correlated to dry cell weight by centrifuging a known volume of fermenter broth, recovering the cells, washing once in saline, re-centrifuging, and weighing the cells after drying in a  $110^\circ\text{C}$  oven. Microbial doubling times were determined by direct measurement of optical density at 640 nm.

The product organic acids were measured by gas chromatography. The volume of gas produced was monitored by a wet test meter and the composition ( $\text{CO}_2/\text{H}_2$ ) of the gas in the head space was determined by gas chromatography. The percent conversion of sucrose was calculated by the weight of products detected.

The nutrient medium employed is described in Table 3.2. In some experiments the yeast extract was omitted. Fresh medium was always bubbled for 30 minutes with  $\text{N}_2$  before addition to the fermenter. Wasting of fermenter broth was always done under a  $\text{N}_2$  atmosphere.

### 3.5 Pretreatment Experiments

To improve the digestibility of some substrates, a variety of pretreatments were tested. The pretreatments were performed at acid, alkaline, and neutral pH and at temperatures ranging from ambient to  $175^\circ\text{C}$ . Comparison of results has indicated the most promising pretreatments for use in the process.

Acid pretreatment were performed on slurries containing 3-5% (w/v) of substrate in 0.6% acetic acid. The treatments were done in high pressure Parr® reactors so that temperatures up to  $175^\circ\text{C}$  could be achieved. The pretreatment time varied. In some experiments, HCl was used instead of acetic acid.

Neutral pretreatments were done in the same manner as the acid pretreatments without addition of acetic acid. This type of pretreatment is known as autohydrolysis.

The alkaline pretreatment used has been described by Rathin Data of Exxon.\* Solutions were made alkaline with 0.5% (w/v)  $\text{Ca}(\text{OH})_2$  and

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\* Biotech. Bioeng. 23: 61-77 (1981)

Table 3.2

Composition of Stirred-Fermenter Medium\*

<u>Salt</u>	<u>Concentration (g/l)</u>
Na <sub>2</sub> HPO <sub>4</sub>	0.17
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	1.59
K <sub>2</sub> HPO <sub>4</sub>	0.44
MgSO <sub>4</sub> · 7H <sub>2</sub> O	0.53
KCl	0.08
Yeast Extract	1.0
12 N HCl	1.5 ml

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\* From Torre&Goma in Biotech. Bioeng. 23:185-199 (1981).

0.66% (w/v)  $\text{Na}_2\text{CO}_3$ . Treatments were for two hours to three days at temperatures between 37 and 150°C.

After pretreatment, the slurries are cooled, neutralized, and 2-bromoethane sulfonic acid and  $\text{CaCO}_3$  are added. The fermentations are then inoculated and performed as described in Section 3.1.

The effect of pretreatment on dissolved sugars was also tested. After pretreatment of a sample containing a known amount of dissolved glucose, the reducing sugar level was measured by a phenol-sulfuric acid assay.\* The disappearance of dissolved reducing sugars over time was documented.

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\* Dubois et al. Anal. Chem. 28:350 (1956).

## Section 4

### EXPERIMENTAL RESULTS

#### 4.1 Digestibility of Potential Substrates

Digestibility experiments were carried out in static flask fermenters on all biomass forms as described in Section 3.1. Conversion to products is reported as percent of total solids charged to the fermenter. Calculations of conversions are based on organic acid concentrations present in the fermenter broth as measured by gas chromatography. Plots showing conversion of substrate as a function of time are presented in Figures 4.1 - 4.5. Table 4.1 lists conversions for untreated substrates.

Pseudo-first-order rate constants were calculated from batch experimental data based on the following equation:

$$- \ln C_A/C_{AO} = kt$$

where  $C_{AO}$  is initial substrate concentration,  $C_A$  is substrate concentration at time  $t$ ,  $k$  is the first-order rate constant in  $\text{days}^{-1}$ , and  $t$  is elapsed time in days. Figures 4.6 to 4.10 are plots to determine rate constants for a number of substrates, and Table 4.2 lists measured rate constants.

High conversion efficiencies were achieved without pretreatment for the marine algae Chondrus crispus (>90% of volatile solids), starchy substrates such as corn meal, and high sugar substrates such as molasses and sweet sorghum. Pretreated wood substrates from Iotech and wood liquors from Mississippi State University were also converted to product in high yields. Once the cellulose and hemicellulose sugars become accessible to the microorganisms, they are metabolized by the organisms and product acids are produced. In low-lignin materials such as corn starch or Chondrus crispus, the sugars are readily available for use by the microorganisms. In substrates such as wood chips, the native ligno-cellulosic structure must be disrupted before conversion of the sugar polymers will proceed. The glucose portion of Iotech pretreated Aspen was 78% converted to products.

Figure 4.1

SAMPLE 011545 - CONVERSION OF SWEET SORGHUM

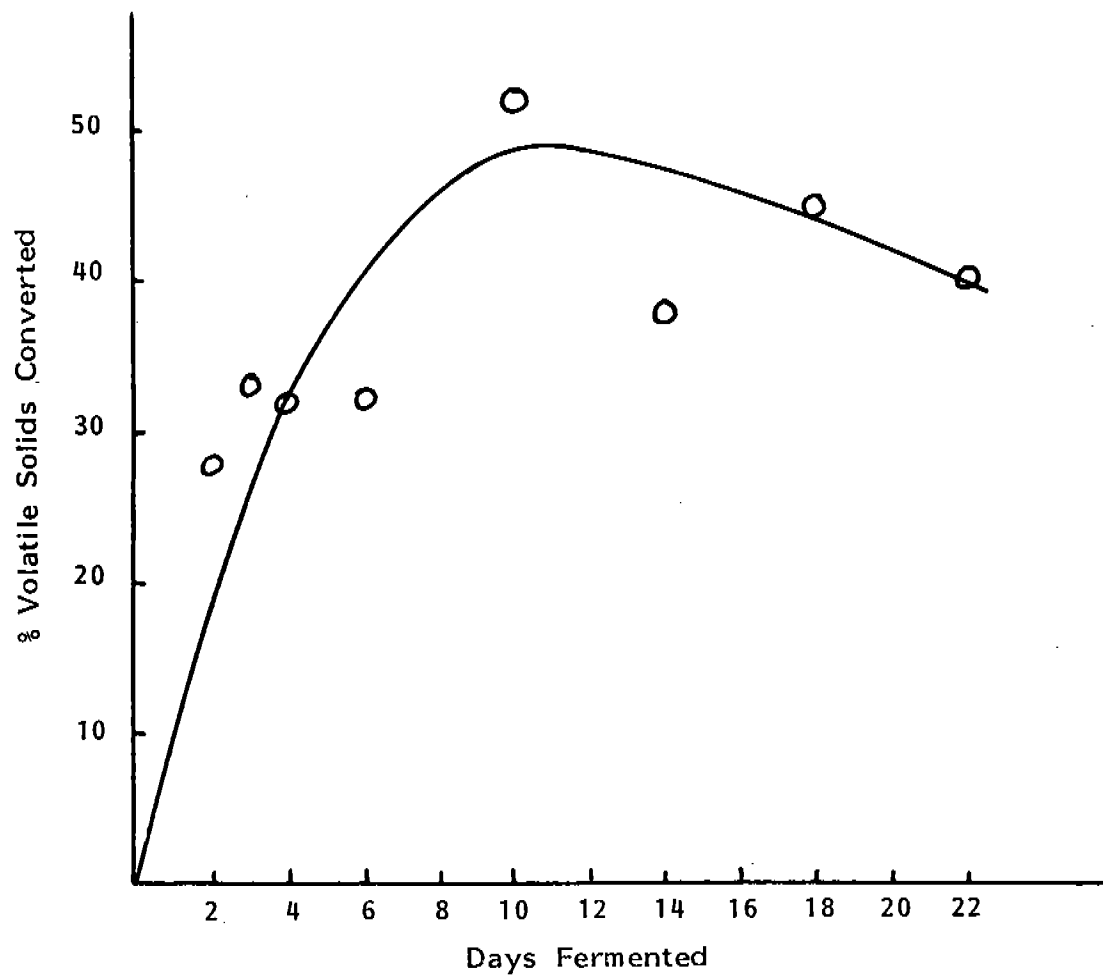


Figure 4.2 ) Fermentation of Molasses (# 07236)

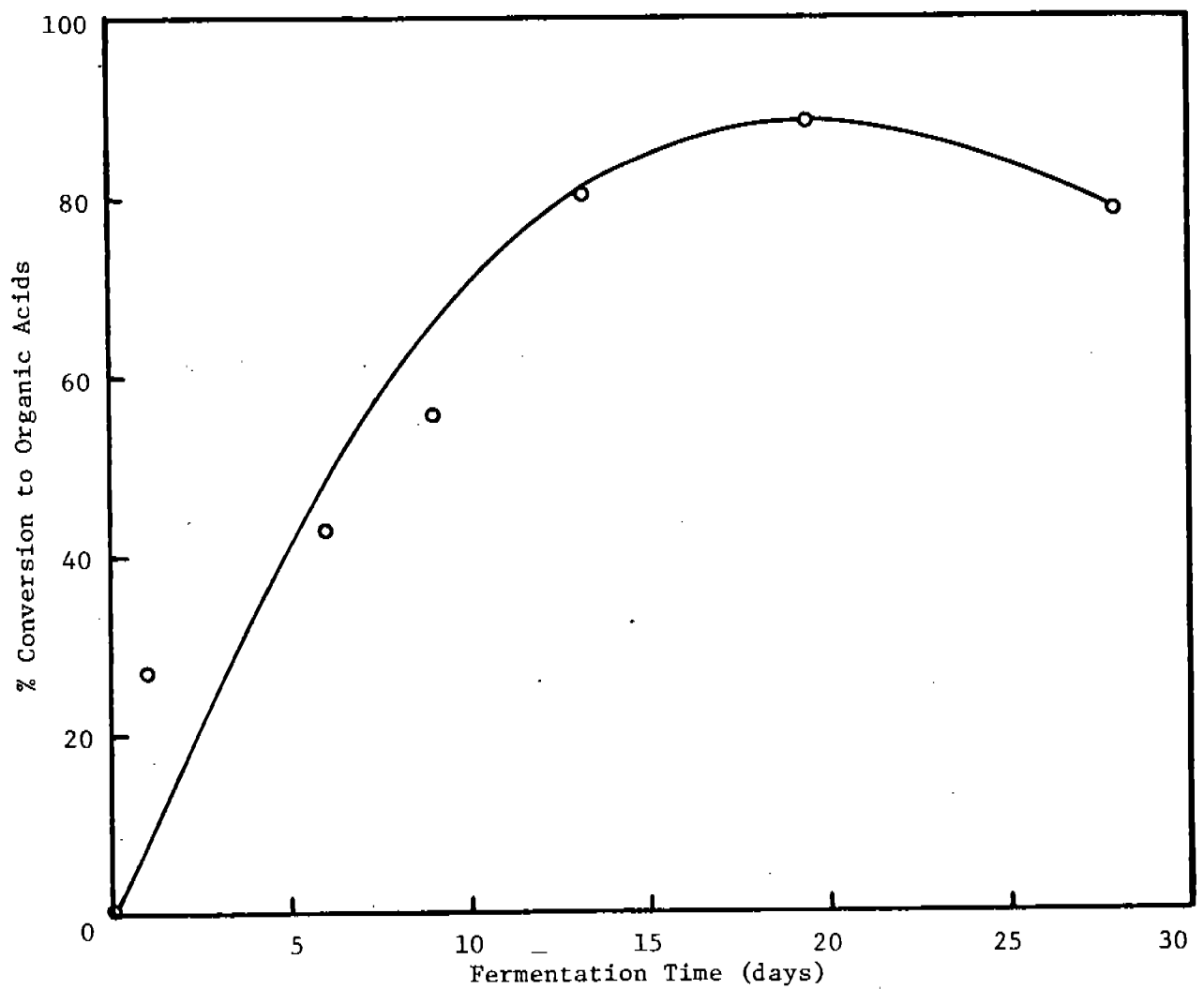


Figure 4.3  
Conversion of Corn Meal to Organic Acids

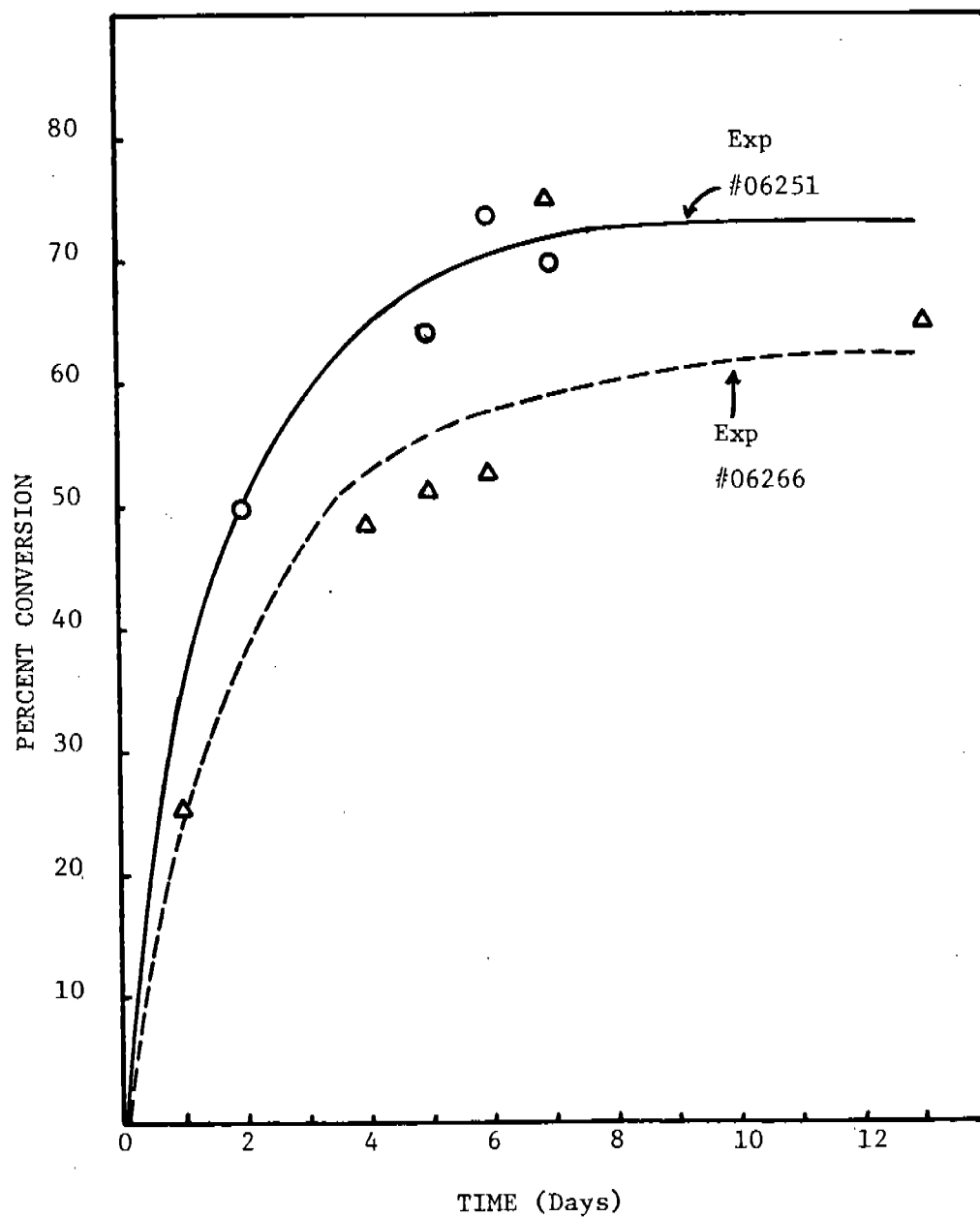




Figure 4.4

FERMENTATION OF GROUND AND UNTREATED  
CORN KERNELS  
(#08075)

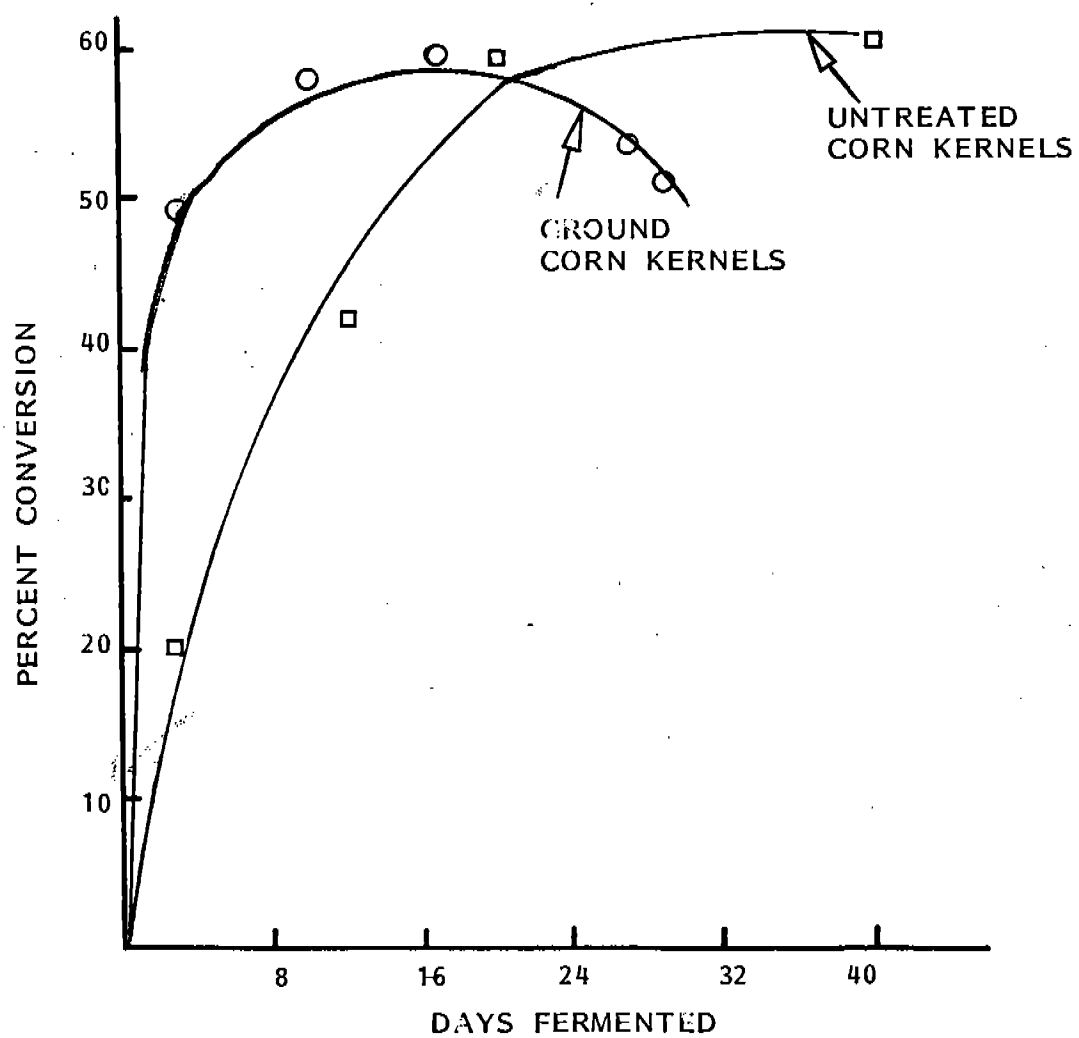


Figure 4.5

CONVERSION OF ASPEN TO ACIDS

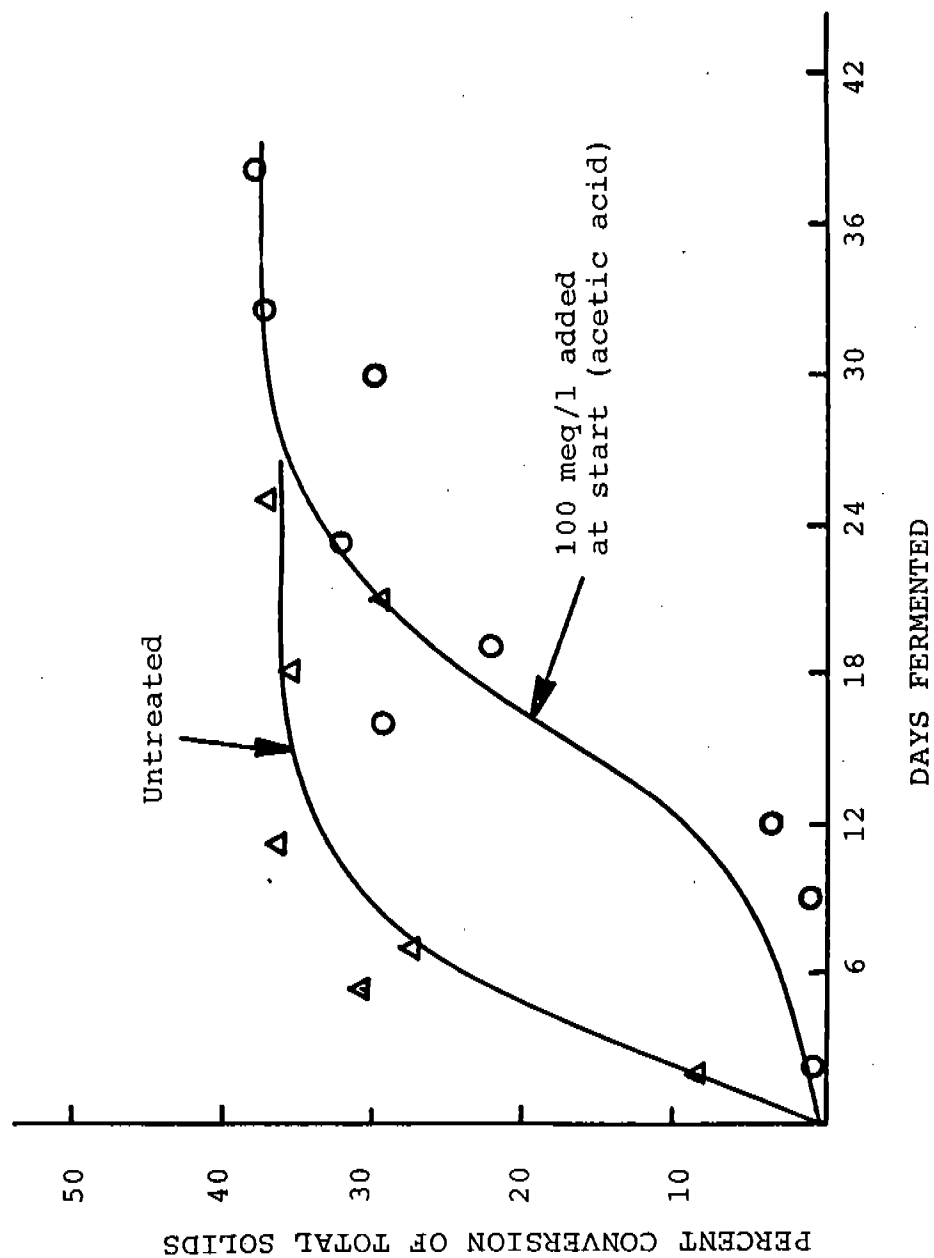


Table 4.1

## Conversion of Untreated Substrates to Organic Acids

<u>Substrates</u>	<u>% Conversion of Total Solids</u>
Water hyacinth	20
Wheat straw	21
Alfalfa	21
Corn meal	82
Kudzu	15
Ragweed	20
Sunflower plants	22
Hydrolysis-grade Aspen	33
Corn stover	10
Sweet sorghum	45
Sta-bl-cob®	22
Corn starch	73
Molasses	85
Corn kernels	65
Bagasse (sugar cane)	16
Lite-R-Cob®	37
Feed-grade Aspen	10
<u>Hydrilla</u>	30
Pine liquor -320°F (Mississippi State)	40
Pine liquor -300°F (Mississippi State)	60
Oak liquor -320°F (Mississippi State)	55

Figure 4.6  
FIRST-ORDER RATE PLOT OF A SWEET SORGHUM  
FERMENTATION

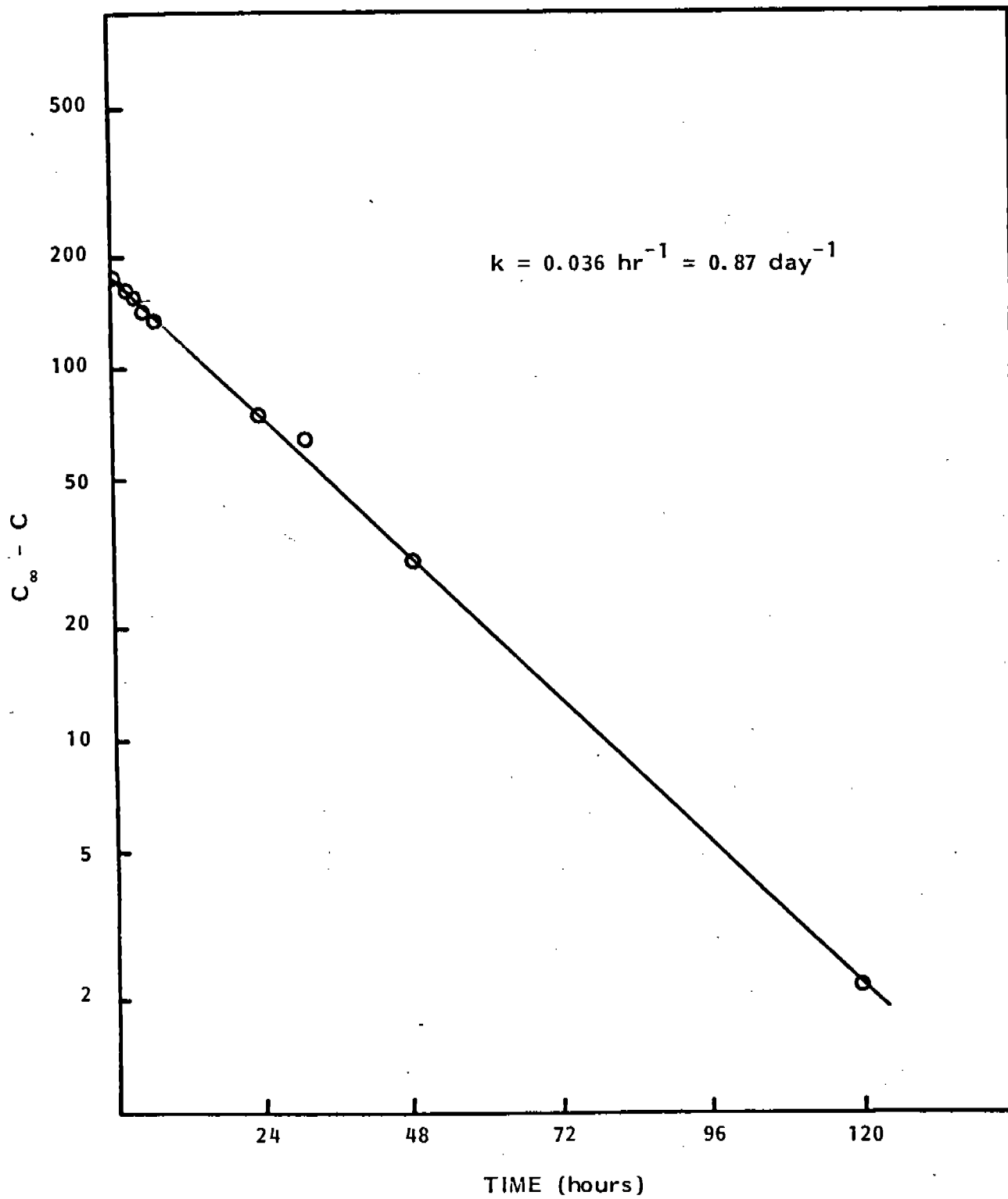


Figure 4.7  
FIRST-ORDER RATE PLOT FOR CORN STARCH  
(#011926-A)

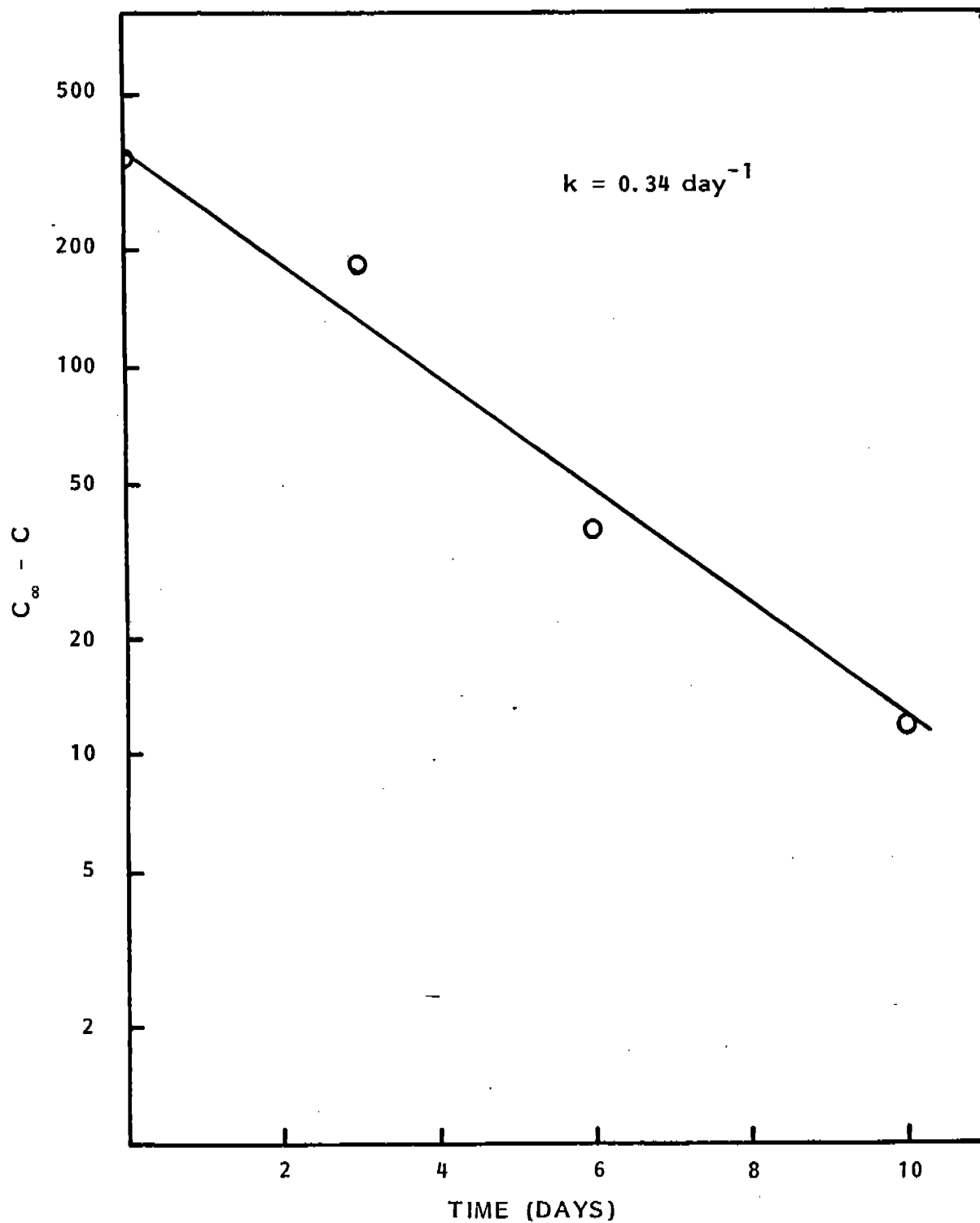


Figure 4.8  
FIRST-ORDER RATE PLOT FOR UNTREATED SOLKA-FLOC  
(#012315)

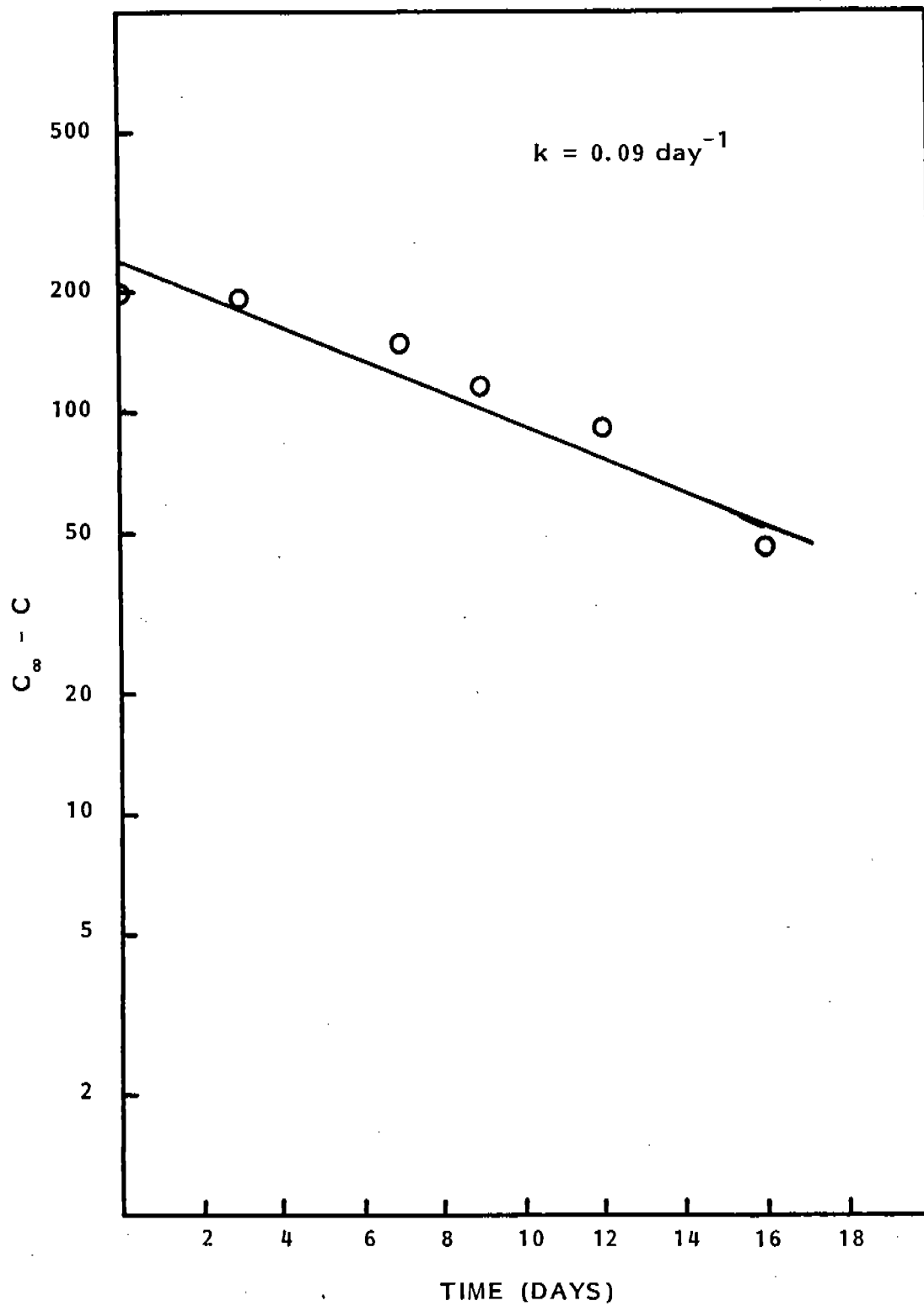


Figure 4.9  
FIRST-ORDER RATE PLOT FOR PRETREATED CORN STOVER  
(#011759)

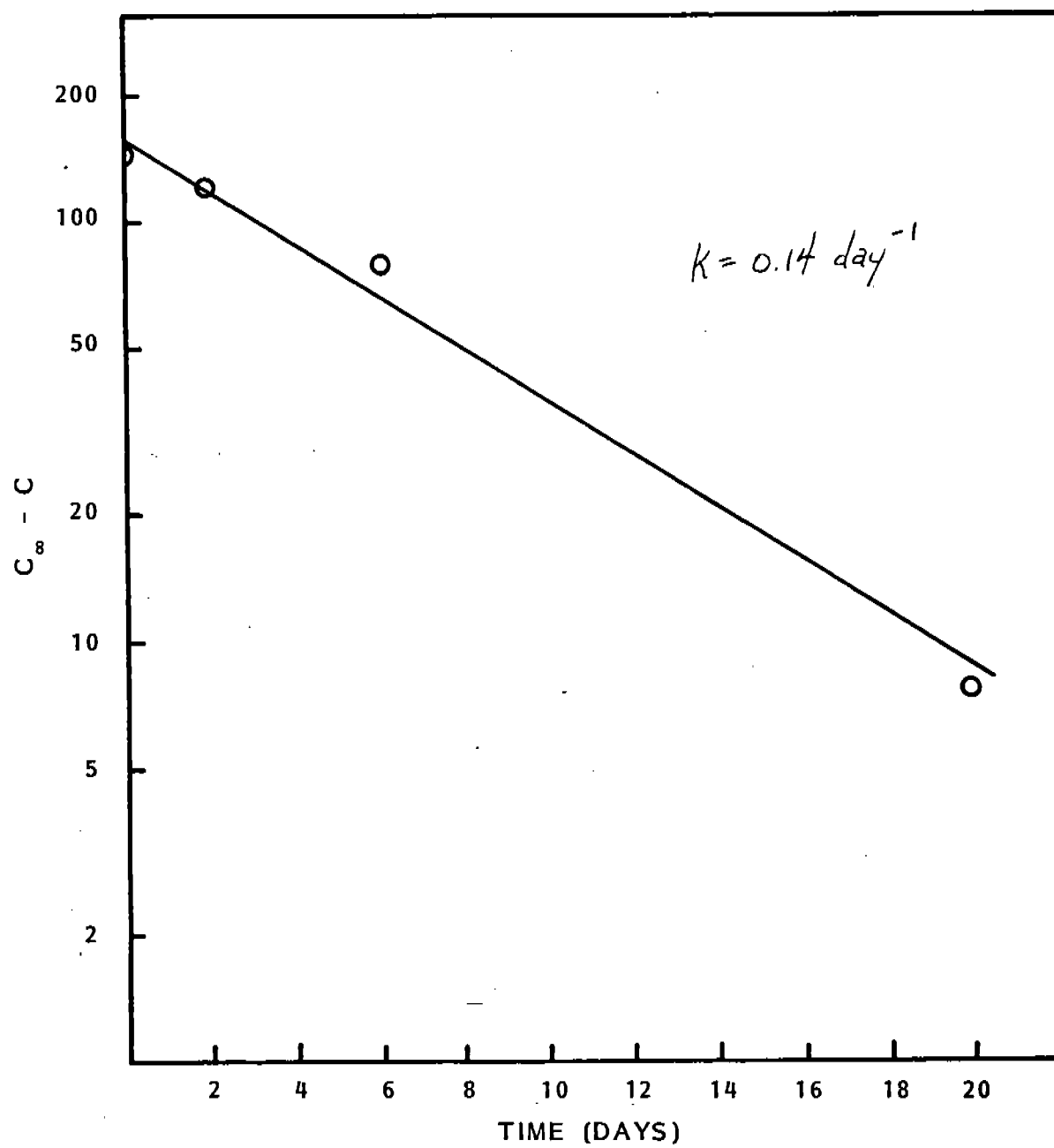


Figure 4.10

FIRST ORDER RATE PLOTS  
FOR CONVERSION TO ACIDS

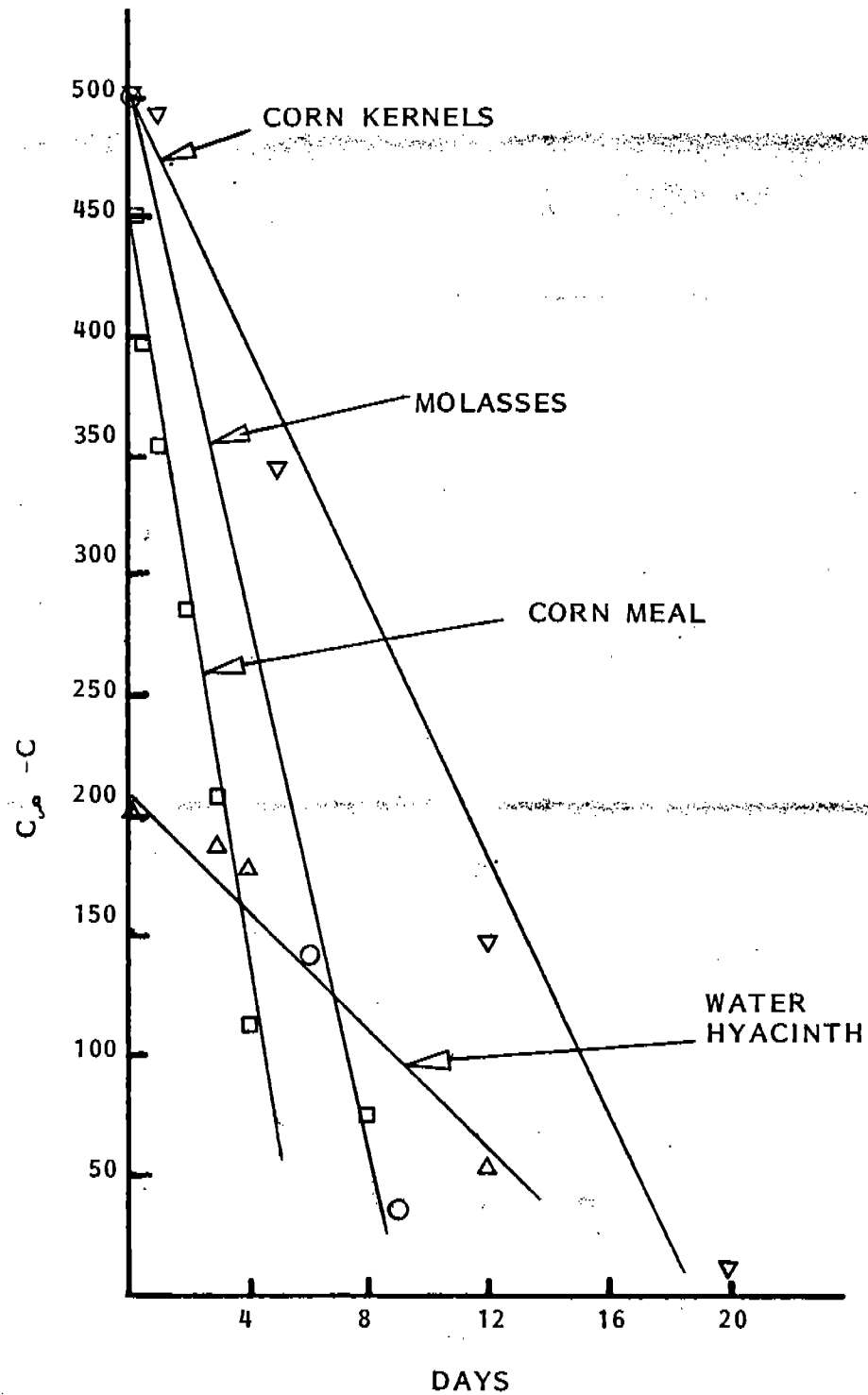




Table 4.2

## First-Order Rate Constants for Conversion to Organic Acids

<u>Substrates</u>	<u>Rate Constant (day<sup>-1</sup>)</u>
Corn meal	0.26
Iotech Aspen	0.20
Pretreated Corn Stover	0.14
<u>Chondrus crispus</u>	0.77
Alfalfa	0.12
Corn starch	0.34
Solka-floc	0.09
Sweet sorghum	0.21
Corn kernels	0.17
Molasses	0.29
Water hyacinth	0.10

The liquors from pine and oak wet oxidized samples obtained from Gary McGinnis (Mississippi State University) were treated to remove an inhibitory substance before fermentation. The treatment consisted of an alkaline precipitation at pH 12 followed by filtration through 0.5 $\mu$  Millipore® filters and neutralization. Conversions of the dissolved sugars in these fermentations approached quantitative levels, leaving the dissolved lignin degradation products behind.

First order rate constants generally reflect the ease with which suspended substrate is hydrolyzed and solubilized by the micro-organisms. Rate constants calculated from static flask digesters run in batch mode are listed in Table 4.2. Starch is more readily solubilized than cellulose as reflected by the rates observed for corn starch and Solka-floc®. Accessibility to the bacteria is also important and may be the cause for lower rates observed for corn kernels. The conversion rate of cellulose in Iotech Aspen is greatly enhanced by the pretreatment employed. The carbohydrate portion of Chondrus crispus is the most easily digested and exhibits the highest rate constant.

#### 4.2 Substrate Composition and Degradability

Compositional analysis to determine ash, lignin, cellulose and hemicellulose, and toluene-ethanol soluble fractions was performed on substrates and fermentation residues as described in Sections 3.2 and 3.3. Heats of combustion were also measured. This data has allowed assessment of potential conversion efficiencies and prediction of the most easily convertible substrates.

The results of analyses of fresh substrates and residues are listed in Table 4.3. The sugar polymer fractions, and in some cases the toluene-ethanol soluble portion, are convertible in the fermentation process. Knowledge of the proportion of these fractions in the substrate allows accurate predictions of maximum conversions to be made.

Table 4.3  
Composition of substrates and Residues

Substrates	TOLUENE - ETHANOL EXTRACT (%)	ACID HYDROLYZED (%)	ASH (%)	LIGNIN (%)	SUGAR ASSAYED (%)
Corn stover	1.5	68.9	7.1	22.5	--
Aspen (Hydrolysis Grade)	21.0	51.0	0.0	28.0	43.0
Aspen (Feed Grade)	20.0	64.0	0.0	16.0	49.0
Sweet sorghum	24.6	48.6	1.2	22.8	20.3
Lite-R-Cobs	7.8	81.0	1.2	10.0	46.0
Sta-B1-Cobs	7.2	81.0	1.8	10.0	50.0
Aspen (Hyd. Grade) Residue	21.3	60.0	4.0	14.7	15.8
Sweet sorghum residue	2.6	73.2	4.0	20.2	15.2
Corn stover residue	14.6	56.7	7.7	21.0	--

Comparison of the composition after fermentation with that of the fresh substrate allows some conclusions to be made. Hydrolysis grade aspen differs from its fermentation residue in the glucose detected by HPLC. When the recovered weight is taken into account, almost 80% of the glucose has been converted. The decrease in lignin content of the residue may be due to solubilization of some of the lignin during the fermentation. The corn stover residue is from a fermentation which was 45% converted to product after alkaline pretreatment. The increase in the toluene-ethanol extract is not easily explained, but the nature of the substrate may have been altered significantly by the pretreatment. The presence of a large acid hydrolyzable portion indicates the potential for even higher conversion. A portion of the toluene-ethanol extract in sweet sorghum is sucrose. This accounts for the high percent change in the amount detected in the residue, since all the sucrose is converted to acids. The acid hydrolyzable portion in sweet sorghum is largely cellulose from the stem of the plant, not detected as monomer sugars and not easily fermented to products. Pretreatment should help to convert this portion, but care must be taken not to degrade the sucrose during the pretreatment.

Heats of combustion of substrates and residues have also been measured and are listed in Table 4.4. Substrates with a high lignin content have elevated heats of combustion, and their residues will have even higher heats of combustion. The residues of substrates with high ash content will have the lowest heats of combustion. The heat content of the residue will determine the value of recovery for use as a fuel.

#### 4.3 Rate of Sucrose Conversion

The rate of conversion of sucrose under anticipated operating conditions for this process was measured in a chemostat fermenter. The first-order rate constant obtained from these experiments provides an upper limit for conversion rates of any chosen substrate. These experiments also helped to define nutrient requirements for the particular mixed culture anaerobes present in these fermentations.

Table 4.4

## Heats of Combustion of Substrates and Fermentation Residues

<u>Substrates</u>	<u>Btu/lb TS</u>
Wheat straw	7817
Alfalfa	7619
Corn meal	7663
Ragweed	7440
Sunflower Plants	6598
Aspen (Hydrolysis Grade)	8833
Corn stover	7680
Sweet sorghum	6935
Sweet sorghum residue	7839
Corn kernels	8044
<u>Hydrilla</u>	7025
Bagasse	7791
Pine liquor -300°F (Mississippi State)	7156
Pine solids -300°F (Mississippi State)	8467
Pine liquor -320°F (Mississippi State)	6935
Pine solids -320°F (Mississippi State)	8582
Oak liquor -320°F (Mississippi State)	6909
Oak solids -320°F (Mississippi State)	8597
Corn stover residue	9025
Aspen (Hydrolysis Grade) residue	8250
Alfalfa residue	6738
Sunflower residue	6898
Ragweed residue	7996
Aspen (Feed Grade)	8750
Rice straw	6800
Barley straw	7540

The fermenter was operated at constant retention times (1.5 to 3.0 days) until daily acid and cell production reached steady-state levels. Then doubling times for the cells were measured after diluting 200 ml of culture with 2.8 liters of fresh, degassed medium. The doubling time was measured under three conditions. Initially, no nutrient supplement was added to the medium. A doubling time between eight and ten hours was observed, and no period of logarithmic growth was achieved. These results were interpreted to mean that the culture was nutrient-limited. To overcome this limitation, a second doubling time experiment was performed with broth from the 240-liter Chronodrus crispus fermentation added to the medium. No changes in the growth characteristics of the bacterial culture were observed in these experiments. In the third attempt to measure doubling times, 1 g/l Difco yeast extract was added to the medium. In these experiments, the steady-state acid and cell yield of the reactor increased. The growth curve exhibited a period of logarithmic growth when the doubling time was 1.5 hours. The volumetric rate of product formation was over 8 milli-equivalents/liter/hour. A correlation was made between optical density and dry cell weight. The conversion factor was determined to be 0.37 grams cells/liter/absorbance unit at 640 nm.

First-order rate constants were measured with initial concentration of sucrose in the range of 12-20 g/l. The data from the fermenter (acid and gas production) were treated as batch data over periods of 8 or 16 hours. Rate constants and fermenter conditions are shown in Table 4.5. The mean value for the rate constant calculated from 5 experiments is  $1.67 \pm 0.32 \text{ days}^{-1}$ . This represents the maximum achievable rate under the conditions employed in the fermentation (pH, organic acid level, nutrient additions). It provides an upper limit to rates achievable for other substrates. The rates reported in Section 4.1 for suspended substrates most likely represent the relative difficulty of hydrolysis and solubilization for each material. Rates, therefore, may be increased in the presence of proper nutrients by treating the substrates in such a manner as to render them more easily solubilized. Exploration of methods of increasing the ease of solubilization has been begun with investigation of pretreatments and will be continued in the future.

Table 4.5  
Conversion Rate of Sucrose in Continuous-Stirred Fermenter

<u>ELAPSED TIME (HRS)</u>	<u>TOTAL ACID CONCENTRATION (meq/l)</u>	<u>FINAL CELL DENSITY (g/l)</u>	<u>FIRST ORDER RATE CONSTANT (DAY<sup>-1</sup>)</u>
16	60.3 - 172.9	3.16	1.41
8	57.6 - 133.1	—	1.55
16	44.4 - 172.6	3.14	2.22
8	57.5 - 117.9	—	1.57
16	39.3 - 131.7	2.68	1.58

Mean rate constant:  $1.67 \pm 0.32$  (5)

#### 4.4 Effect of Pretreatment on Conversion

A variety of pretreatments have been attempted in an effort to increase conversions of substrates to organic acids. Two types of pretreatment have been extensively investigated - at acid or alkaline conditions. Acid hydrolysis in 0.6% acetic acid at temperatures between 100 and 175°C for two hours showed little or no improvement of conversion efficiencies for most substrates tested. Hydrilla was an exception (Figure 4.11), exhibiting up to 10 percent improvement in conversion after autohydrolysis at 175°C.

Alkaline pretreatment was more successful. Extensive experiments were performed on corn stover to optimize pretreatment parameters. Only a limited advantage was seen at elevated temperatures. In fact, at higher temperatures dissolved sugars were degraded, and conversion by fermentation of these sugars was reduced to 70 percent of theoretical. The effect of various times and temperatures on alkaline pretreatment of corn stover is shown in Figure 4.12. The treatment chosen for most other substrates, particularly those containing soluble sugars, is alkaline soaking at ambient temperature for 2-4 days. For substrates without soluble sugars (wheat straw, corn stover, etc.) shorter pretreatments at higher temperatures may be preferable. The effect of various pretreatments on different substrates is listed in Table 4.6.



Figure 4.11

AUTOHYDROLYSIS OF HYDRILLA

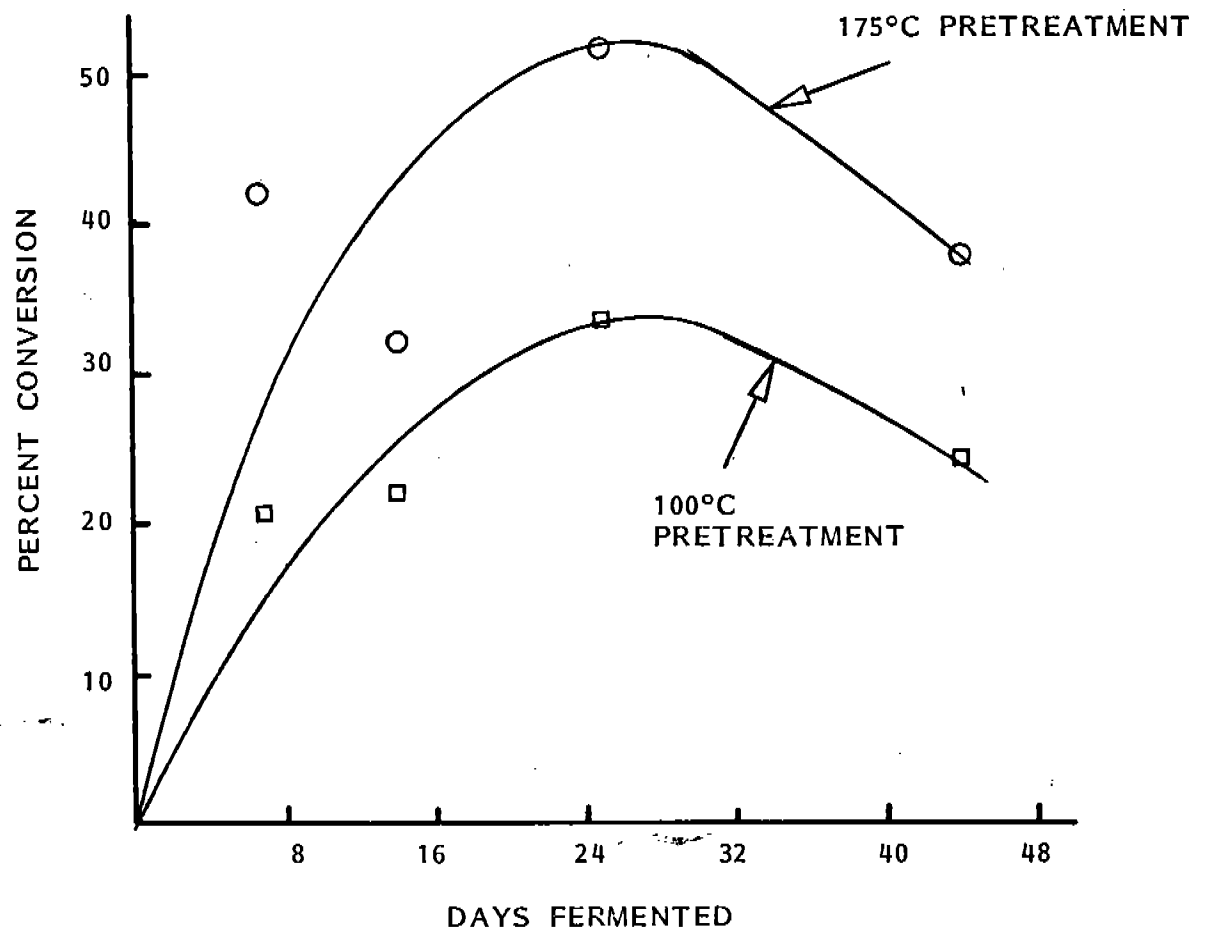


Figure 4.12

EFFECTS OF ALKALINE PRETREATMENT  
ON DIGESTIBILITY OF CORN STOVER

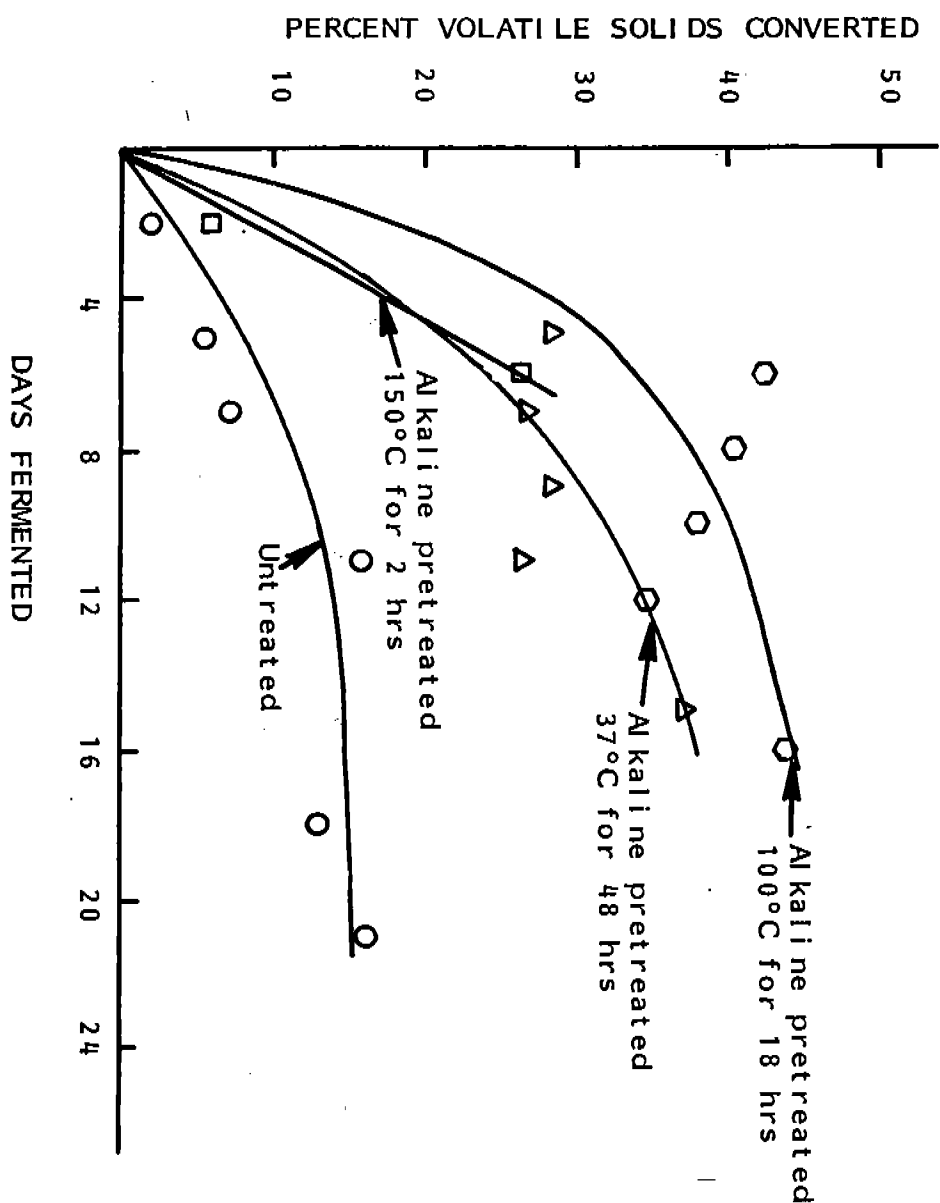


Table 4.6

## Conversion to Organic Acids After Pretreatment

<u>Substrate</u>	<u>Pretreatment</u>	<u>Percent Conversion (TS)</u>
Water Hyacinth	0.6% Acetic; 175°C - 2 hrs	29
Kudzu	0.6% Acetic; 175°C - 2 hrs	16
Wheat Straw	0.6% Acetic; 175°C - 2 hrs	33
Cattail roots	0.6% Acetic; 175°C - 2 hrs	26
<u>Hydrilla</u>	0.6% Acetic; 175°C - 2 hrs	30-50
<u>Hydrilla</u>	0.6% Acetic; 100°C - 2 hrs	30-50
Alfalfa	0.6% Acetic; 175°C - 2 hrs	25
Hydrolysis-grade Aspen	0.6% Acetic; 175°C - 2 hrs	38
Corn Stover	0.6% Acetic; 175°C - 2 hrs	14
Corn Stover	Alkaline*; 37°C - 48 hrs	36
Corn Stover	Alkaline; 100°C - 18 hrs	44
Corn Stover	HCl (pH 1); 175°C - 2 hrs	7
Corn Stover	Alkaline; 150°C - 2 hrs	47
Corn Stover	Alkaline; 100°C - 4 hrs	41
Corn Stover	Alkaline; 100°C - 47 hrs	40
Corn Stover	0.6% Acetic; 25°C - 1 hr	8
Sweet Sorghum Residue	Alkaline; 100°C - 48 hrs	10
Sta-bl-cob®	Alkaline; 100°C - 100 hrs	6
Lite-R-Cob®	Alkaline; 100°C - 20 hrs	16
Glucose	Alkaline; 100°C - 100 hrs	68
Feed Grade Aspen	0.6% Acetic; 175°C - 2 hrs	20

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\* Alkaline treatments were in aqueous slurries with 0.5% Ca(OH)<sub>2</sub> plus 0.66% Na<sub>2</sub>CO<sub>3</sub>.

## Section 5

### CONCLUSIONS AND RECOMMENDATIONS

Digestibility experiments have shown that most carbohydrate-containing materials are suitable substrates for the mixed microbial fermentation employed in this process. Some substrates require pretreatment to disrupt the native plant structure, allowing attack by the cellulolytic microorganisms, if substantial conversion efficiencies are to be achieved. Other substrates, particularly starchy materials and marine algae, are easily fermentable without any pretreatment.

Plant material from the three groups of biomass examined were all successfully fermented. Corn, corn meal, and sweet sorghum (crop grown biomass) were all converted in fairly high yields without any pretreatment. Extensive experiments with corn stover (crop residue) indicated it is convertible in reasonable yields (66% of fermentable fraction) after a mild alkaline pretreatment. Initial indications are that this pretreatment is applicable to most of the straw-type crop residues. Forest products (Iotech treated Aspen and pine and oak from Mississippi State University) were also fermentable without additional pretreatment. The hemicellulose portion (solubilized by these pretreatments) as well as the cellulose portion are suitable substrates in these fermentations. Removal of an inhibitory substance by alkaline precipitation was necessary before fermenting the wet oxidized samples (Mississippi State University).

Compositional analysis and kinetic studies have indicated that the cellulosic portion of the biomass is the most difficult sugar polymer to digest. This is most likely due to difficulty in solubilization (hydrolysis) by cellulolytic enzymes, which solubilize starch and pentose polymers more readily. This is particularly apparent from the low rates of conversion measured for cellulosic substrates like Solka-floc and alfalfa.

Appropriate pretreatments of these substrates may be a valuable technique for decreasing residence time and thus digester cost.

The intrinsic rate of sucrose conversion under conditions simulating actual process operation was measured to be significantly greater than any rates measured for non-dissolved substrates. There is strong evidence that the rate-determining step in substrate conversion is the hydrolysis and solubilization. This information gives us a focal point for improving conversion rates. Since rates affect digester size, which strongly affects product cost (capital investment), it is advantageous to pursue operating techniques that will increase conversion rates. It may be best to completely hydrolyze the substrate in a pretreatment before fermentation to organic acids, or some less complete pretreatment to increase the rate of solubilization may be the best alternative. These process variations should be examined as a means of decreasing final product cost.

Future work is planned to continue examining alternate substrates and appropriate pretreatments to obtain maximum conversion efficiencies and rates for each substrate. Compositional analysis will be carried out to help assess the potential of each substrate and the progress made by applying various pretreatments. Promising substrates will be scaled-up from small static flask fermenters to larger chemostat (semi-continuous feed) fermenters to obtain more accurate conversion and rate data. This will be followed by scale-up to our 240-liter continuous feed packed-bed fermenter to provide complete engineering data for pilot plant design. The materials tested, including wood chips, sweet sorghum, and corn stover, all have been shown to be potential substrates for this liquid hydrocarbon fuel process.